ABSTRACT

A novel procedure for performing protein labeling for comparative proteomics termed inverse labeling is provided for the rapid identification of marker or target proteins. With this method, to evaluate protein expression of a disease or a drug treated sample in comparison with a control sample, two converse collaborative labeling experiments are performed in parallel. In one experiment the perturbed sample (by disease or by drug treatment) is isotopically heavy-labeled, whereas, the control is isotopically heavy-labeled in the second experiment. When mixed and analyzed with its unlabeled or isotope light counterpart for differential comparison, a characteristic inverse labeling pattern is observed between the two parallel analyses for proteins that are differentially expressed to an appreciable level. In particularly useful embodiments, protein labeling is achieved through proteolytic ^{18}O-incorporation into peptides as a result of proteolysis performed in ^{18}O-water, metabolic incorporation of ^{15}N (or ^{13}C and ^{2}H) into proteins, and chemically tagging proteins with an isotope-coded tag reagent such as an isotope-coded affinity tag reagent.